REMARKS

Reconsideration and withdrawal of all grounds of rejection are respectfully requested in view of the above amendments and following remarks. Applicant's representative thanks Examiner Vogel for courtesies extended during a telephone interview on June 25, 2009, the substance of which has been incorporated into the following remarks.

1. As discussed during the interview, enclosed is a certified copy of the priority document and a certified translation of the priority document, which has been described as true and accurate by Dr. Okuhara. This is being submitted to antedate the reference to Ninomiya et al. (PNAS, 101, 33, 12248-12253 (2004). Withdrawal of the rejection of claims 1-5, 9, 10, 14-17 in view of Ninomiya is respectfully requested.

2. Claims 1-5, 9, 10 and 14-18 were rejected under 35 U.S.C. 103 in view of Hooykus et al. (patent application Pub. No. 2004/0073967, "Hooykus") in view of Ninomiya.

As discussed during the interview, overcoming Ninomiya as a reference defeats this rejection. In this regard, it is noted that Examiner Vogel stated that she would conduct an updated search.

Hooykus has deficiencies as a primary reference. The experimental data disclosed in Hooykus is relevant to budding yeast. In terms of systematics budding yeast are eukaryotes. However, the recombination mechanisms of such budding yeasts largely differ from the recombination mechanisms of other types of eukaryotes. Persons skilled in the art recognize this fact as common technical knowledge. It has been known that the "recombination" of eukaryotes is broadly divided into the categories of "homologous recombination" and "non-homologous recombination." In the case of budding yeasts, approximately 90% of the "recombination" is "homologous recombination" and their homologous recombination rate is originally high (Mutation research 566;131-167, 2004, see p. 134, 3.2 Homologous recombination in *Saccharomyces cerevisiae*). On the other hand in the case of eukaryotes

other than budding yeasts, such as *Neurospora crassa* disclosed in the present specification, their "homologous recombination" rate is extremely low (from several percent to approximately 20%); (please refer to Table 2 in the present specification relating to the homologous recombination frequency of wild type strains).

As discussed above, the idea that the experimental results for budding yeasts, which are organisms having originally high recombination efficiency, cannot be applied to the homologous recombination of other types of eukaryotes such as *Neurospora* or *Aspergillus* disclosed in the present application, is common technical knowledge in the present technical field. Therefore, the present invention is not obvious in view of Hooykus.

During the interview it was pointed out that the increased rate of homologous recombination with regard to wild type is much greater as a result of the present invention than in Hooykus. Table 2 of Hooykus shows that the frequency of illegitimate recombination (IR) or non-homologous recombination in the wild type budding yeast (YHP25)) is 1.6×10^{-7} and the frequency of homologous recombination (HR) is 2.4×10^{-5}. The percentage of homologous recombination in such wild-type budding yeast can be calculated to be approximately 99% using these numerical values ((Frequency of HR + frequency of IR) X 100). This indicates that the change in the percentage of homologous recombination frequency from the wild type strain via the method of Hooykus is only 1% at most. Therefore in comparison with the method described in Hooykus, the inventive method of conducting homologous recombination achieves homologous recombination extremely efficiently.

From the data of Table 2 of Hooykus, homologous recombination efficiency is hardly increased, if at all, in the KU70-disrupted strain. Table 2 of Hooykus shows that the homologous recombination frequency (Freq of HR) of wild type budding yeasts (YPH250 (WT)) is 2.4×10^{-5}, whereas the homologous recombination frequency (Freq. of HR) of

KU70 deletion mutants (yku70) is 3.3 x 10^{-5}. These results show that the homologous recombination rate is hardly increased, even though KU70 is deleted. At most there is an increase of approximately 1.3 times.

These differences are featured in new claims 19 and 20. Claim 19 states the following:

wherein a rate of said homologous recombination of said filamentous fungi in which there is said decrease or loss of the functions of the gene is increased by at least a factor of 5 compared to a rate of homologous recombination of a wild type of said filamentous fungi.

Claim 20 is the same as claim 19 except the increase is by at least a factor of 12.5.

In this regard, the present application (e.g., Table 2 on p. 17) discloses that the homologous recombination rate of wild type *Neurospora crassa* is 19% whereas the homologous recombination rate of KU70 deletion mutants (ncku70) is 100% and thus, the homologous recombination rate is increased by a factor of approximately 5. Moreover, section "4. Disruption efficiency of any given gene-kexB" on p. 24 of the present specification discloses that the homologous recombination rate of wild-type *Aspergillus nidulans* is 7.2% whereas the homologous recombination rate of KU70-deleted strains is 90% and thus, the homologous recombination rate is increased by a factor of approximately 12.5. For these reasons it is submitted that claims 1 and 19, and their dependent claims, are not obvious in view of Hooykus.

3. Claims 1-5, 9, 10 and 13-18 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement.

Applicant's representative appreciates Examiner Vogel's comment that the amended claims were well received with regard to overcoming this rejection. Examiner Vogel noted that Applicant should discuss how one of ordinary skill reading the present disclosure would be able to induce the decrease or loss of function of the genes LIGIV and XRCC4 (e.g., by mutating or deleting the genes) in filamentous fungi without undue experimentation in view

of the focus in the present application on the KU70 and KU80 genes. A discussion of LIGIV and XRCC4 genes involved in non-homologous recombination that may have a decrease or loss of function induced therein, is provided, for example, on page 5, third full paragraph of the specification and in claim 5. See pages 7 and 8 of the specification, the section Best Mode for Carrying out the Invention, for a discussion of how to identify and use homologous genes of interest. At the time of the priority application the LIGIV and XRCCIV genes were sequenced in humans; and *Neurospora* and *Aspergillus* genomes were also sequenced. Through BLAST searching or the like homologous genes in filamentous fungi could have been identified and used in the invention. This would require no more than ordinary skill in the art as such identification is common practice. In fact, filed concurrently herewith is a Declaration by the inventor, Hirokazu Inoue, describing an experiment in which he asserts he used no more than the knowledge he had at the time of filing the priority application in inducing a decrease or loss of function in XRCC4 and LIGIV genes resulting in improved rates of homologous recombination.

More specifically, genes necessary for non-homologous recombination are limited to 4 genes, namely, KU70, KU80, LigIV and XRCC4. It has actually been confirmed without undue experimentation that the homologous recombination rate is increased in LigIV-deficient *Neurospora crassa* and *Aspergillus oryzae* strains and in a XRCC4-deficient *Neurospora crassa* strain. This is shown by the attached Declaration by the inventor in experiments conducted after the filing of the present application.

At the time of filing the present application, the sequence information of human LigIV (Accession No. X84331; registration date December 13, 1994) and the sequence information of human XRCC4 (Accession No. BAB20668; registration date September 2, 1998) were already known. At that time, genome sequencing of Neurospora (http://www.broad.mit.edu/annotation/genome/neurospora/Home.html) and Aspergillus

(http://www.broad.mit.edu/annotation/genome/aspergillus_group/Multihome.html) were completed. Thus, at the time of filing the present application one of ordinary skill in the art could have applied a method such as BLAST searching or the like on the basis of the sequence information of human LIGIV or the sequence information of human XRCC4 (disclosed from p. 7, line 18 to p. 8, line 17 of the present specification), so as to obtain the gene information of LIGIV of *Neurospora crassa* and *Aspergillus oryzae*, and the gene information of XRCC4 of *Neurospora crassa*, for example. The enclosed Declaration describes the results of experiments performed based on the gene information of LigIV and XRCC4 of *Neurospora crassa* and the gene information of LigIV of *Aspergillus oryzae*, which were obtained by the aforementioned method.

Table 1 of the enclosed Declaration shows the results obtained by comparing the prtR gene targeting rate of the LIGIV gene (ligD)-disrupted strain (ΔligD) of *Aspergillus oryzae* with the gene targeting rate of the wild-type strain (NS4) of *Aspergillus oryzae*. Tables 3 and 4 show the results obtained by comparing the os-2 gene targeting rate (Table 3) and the as-3A gene targeting rate (Table 4) of the LIGIV gene (ncLig4)-disrupted strain (ΔncLig4) of *Neurospora crassa* with those of the wild-type strain of *Neurospora crassa.* The results shown in Tables 1, 3 and 4 demonstrate that the LIGIV-disrupted strain of *Aspergillus oryzae* exhibited a homologous recombination rate that was approximately 11 times higher than that of the wild-type strain, and that the LIGIV-disrupted strain of *Neurospora crassa* also exhibited a homologous recombination rate that was approximately 5.7 times higher than the wild-type strain. Table 5 shows the results obtained by comparing a mtr gene targeting rate in an ncLIF1-disrupted strain of *Neurospora crassa* (yeast LIF1 being a homolog of human XRCC4) compared to that of a wild type strain of *Neurospora crassa.* The results of Table 5 show that homologous recombination occurred in the ncLIF1-disrupted strain of *Neurospora crassa* at a rate that was 5.7 times higher than in the wild type strain of *Neurospora crassa.*

A patent application is not intended to be a production document but is only required to teach one of ordinary skill in the art how to make and use the invention and this must be in such clear terms that it is apparent that the inventor was in possession of the claimed invention when the application was filed. One of ordinary skill in the art would have been able, without undue experimentation, to identify the LIGIV and XRCCIV genes of filamentous fungi belonging to the *Neurospora* or *Aspergillus* species, based on the sequenced human counterpart genes and sequenced *Neurospora* and *Aspergillus* genomes, and appropriate techniques like BLAST searching (in fact this is what was done in the Declaration using the information available at the time of the priority application) and then could have induced a decrease or loss of function of these genes as claimed and conducted homologous recombination as claimed, following the description in the application with regard to the KU70 and KU80 genes. The experimental results of the Declaration confirm that the LIGIV and XRCC4-deficient strains had the same effects as those of the KU70 and KU80-deficient strains. From the above it can be said that, as of the time of filing the present application, the inventor had already achieved the invention disclosed in the present application regarding at least KU70, KU80, LIGIV and XRCC4 of *Neurospora crassa*, KU70 and KU80 of *Aspergillus nidulans* and LIGIV of *Aspergillus oryzae*.

The Examiner requested that Applicant provide references showing that the LIGIV and XRCC4 genes are conserved from filamentous fungi to humans. Enclosed are three references published before the priority date that are submitted to show that there was sufficient conservation of these genes from filamentous fungi to humans to enable one of ordinary skill in the art to identify and to at least induce a mutation or deletion in these genes in filamentous fungi.

The authors of Infection and Immunity, vol. 69, No. 1, 137-147 (2001) ("Document 1") cloned LIG4 of *C. albicans* as the yeast and human counterparts of LIG4. They confirm

that LIG4 of *C. albicans* is able to complement a lig4 mutant of *S. cerevesiae* (see, for example, the second paragraph in the left column of page 138). *C. albicans* is categorized as Hyphomytes into which *Aspergillus* is also categorized. Document 1 shows that LIG4 is conserved from filamentous fungi to human.

The paper, Nucleic Acids Research, Vol. 26, No. 24, 5676-5683 (1998), ("Document 2") describes genetic characterization of Dnl4 (fission yeast homolog of human LIG4) based on its homology with mammalian LIG4 (see, for example, the abstract). Document 2 shows that LIG4 is conserved from fungi to mammalian. Therefore, based on the teaching of Documents 1 and 2 one of ordinary skill in the art could have reasonably expected at the priority date of the present application that LIG4 was conserved from fungi or filamentous fungi to humans.

The paper, The EMBO J., Vol. 17, No. 14, 4188-4198 (1998), ("Document 3") reports a function of LIF1, which shares sequence homology with mammalian XRCC4 (see, for example, the abstract). Fig. 2 of Document 3 shows amino acid alignment of yeast LIF1 protein and human XRCC4. LIF4 shares 22% identity and 49% similarity with human XRCC4 (see, for example, lines 11-14 in the second paragraph of the right column on page 4189). Based on the teaching of Document 3, one of ordinary skill in the art could have reasonably expected at the priority date of the present application that XRCC4 is conserved from filamentous fungi (that is belonging to fungi) to humans, since Document 3 shows that fungi XRCC4 (fission yeast is categorized as fungi) is conserved from human XRCC4.

One of the secondary indicia of nonobviousness is copying or use of the invention by others, i.e., effectiveness of the present invention. Many publications have been made after the priority date of the present application in which researchers state the effectiveness of the present invention as attributed to Applicant's work, as shown by the following examples:

1) Mol. Gen. Genomics 275, 460-470 (2006) (Document 4): gene targeting rates of the KU70 and KU80-disruted strains of *Aspergillus oryzae* and *Aspergillus sojae* are increased; "Recently, an increase in the gene targeting frequency of *ku* mutants has been reported in ... *Neurospora crassa* (Ninomiya et al. 2004)";

2) Genetics 172, 1557-1566 (2006) (Document 5): the gene targeting rate of the KU70-disrupted strain of *Aspergillus nidulans* is increased; "Our approach is based on the results of NINOMIYA *et al.* (2004) who found that the deletion of genes required for non-homologous end joining DNA repair (homologs of the human KU70 and KU80 genes) increases the frequency of gene replacement in Neurospora crassa";

3) Eukaryotic Cell, 5, 212-215 (2006) (Document 6): the gene targeting rate of the KU-70 disrupted strain of *Aspergillus fumigatus* is increased; "Recently an advance was made in the molecular biology of the ascomycetous model organism N. crassa by evaluating the effectiveness of gene targeting in a genetic background lacking the nonhomologous end-joining pathway (10): in strains deleted for the Ku70- and Ku80-encoding genes... the relative frequency of homologous recombination is significantly increased..."; and

4) Fungal Genet. Biol. 45, 878-889 (2008) (Document 7): gene targeting rate of the LigIV-disrupted strain of (ligD) of *Aspergillus oryzae* is increased; "However, Ninomiya et al. (2004) have recently demonstrated that deletion of... (homologs of *S. cerevisiae* YKU70 and YKU80, and human KU70 and KU80, respectively) in a filamentous fungi *Neurospora crassa* greatly increases the efficiency of gene-targeting as much as 100%, compared with ~ 20% in wild-type.".

Moreover, the filamentous fungi that is claimed is limited to that belonging to *Neurospora* or *Aspergillus* species. As stated above, the present invention has effects on KU70, KU80, LIGIV and XRCC4 of *Neurospora crassa.* Accordingly, it is clear that the presently claimed invention also has effects on *Neurospora* species which are highly homologous in terms of gene sequence and function.

As described on page 21 of the present application, the inventor has actually obtained the sequence information of KU70 and KU80 genes of *Aspergillus nidulans* based on KU70 and KU80 genes of *Neurospora crassa.* Therefore, the inventor has produced the KU-70 and KU80-disrupted strains of *Aspergillus nidulans* based on the sequence information of the KU70 and KU80 genes of *Aspergillus nidulans*. Thus, the inventor has confirmed that the invention has effects also on such gene disrupted strains (page 24, lines 10-24 of the present specification). Moreover, as stated above, it can be said that the inventor also achieved the invention disclosed in the present application regarding LIGIV of *Aspergillus oryzae*.

The current Office Action is similar to the Office Action in the corresponding Japanese application, and many of the arguments submitted here have been submitted there. As a result, the objections by the Japanese Patent Office have been overcome and a patent has been issued. Reconsideration and withdrawal of all grounds of rejection are respectfully requested in view of the above amendments and the foregoing remarks. Accordingly, an early Notice of Allowance for this application is respectfully requested.

If there are any additional fees resulting from this communication, please charge same to our Deposit Account No. 16-0820, our Order No. WING1-41066.

Respectfully submitted,

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